Studies of the Biology of *Polychoerus carmelensis* (Turbellaria: Acoela)

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LITTLE IS KNOWN about the biology of the Acoela. Hyman has summarized both earlier (1951) and more recent studies (1959: 731 and f.). The Acoela are of particular interest because typically they lack a gut and lack protonephridia, and frequently lack eyes. Thus they carry on a number of biological activities without having the structural modifications associated with these activities. The biology of the Acoela is of further interest because of the hypothesis of Hadzi that the Acoela are the stem group of the Eumetazoa and were derived from ciliates (de Beer, 1954; Hanson, 1958).

Polychoerus carmelensis is found in the pools of the mid-tide horizon in the vicinity of Monterey, California (Ricketts and Calvin, 1952: 49). Costello and Costello have described copulation (1938*a*) and egg laying (1939) in this species.

These studies were conducted at the Hopkins Marine Station of Stanford University, Pacific Grove, California, where the author was studying marine biology as a National Science Foundation science-faculty fellow. I wish to express my appreciation to Dr. L. R. Blinks, director of the Hopkins Marine Station, for providing facilities, and to Dr. Donald P. Abbott for introducing me to *P. carmelensis*.

REACTIONS TO SALINITY

Animals were collected from the tide pools during low tide at Point Pinos and Carmel Point. They were returned to the laboratory and placed in a flat, rectangular glass dish through which sea water (s.w.) flowed. Solutions of 25, 50, 75, 100, 125, and 150 per cent s.w. were made. The dilute solutions were made by mixing sea water from the laboratory pipes with the appropriate amount of distilled water. The concentrated solutions were made by evaporating sea water to form a 200 per cent solution and then diluting this with appropriate amounts of distilled water.

The first experiment was to determine the range of tolerance to salinity. Twenty animals were placed in each of the dilutions of s.w. They were observed for activity every hour during the first 12 hr. and then were checked every 12 hr. for 5 days. Any animals surviving after 5 days were periodically checked for another 2 weeks after which time the experiments were discontinued. Two tests were made for activity. The dishes were shaken gently; healthy animals reacted to this agitation by showing some movement in place or by locomotion. A bright light caused normal animals to locomote. Animals that did not respond to either stimulus were considered inactive until disintegration of epidermal cells was evident, at which time the animals were considered dead.

25 per cent s.w. All animals curled into a U-shaped position immediately upon being placed in the dish. After 1 hr. all animals were dead. The individuals showed a marked swelling and disintegration of the epidermal cells.

50 per cent s.w. Most animals curled into the U-shaped position within 10 min. After 1 hr., only abnormal body movements, characterized by twisting and contractions, were evident. The worms would not attach to the dish, and several extruded material, including copepods that had been engulfed, from the mouth. After 5 hr. 10 animals were transferred to normal sea water. Two hr. later half the worms were active. After 3 hr., 7 of them were active and the other 3 were uncurled. By the end of 16 hr. all animals displayed normal activity. Seven of this group were active 2 weeks later when the experiment ceased.

At 54 hr. 5 of the remaining 10 worms in 50 per cent s.w. were disintegrating and the other 5 showed some slight muscular movement when stimulated. The 5 active worms were transferred to normal s.w., but none of them survived.

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75, 100, 125 per cent s.w. All animals in these concentrations showed normal activity for 2 weeks. After 16 days, 3 animals in 125 per cent s.w. died.

150 per cent s.w. Most animals curled up shortly after being placed in the dish. One hr. later, 3 responded to the light stimulus and 7 responded upon being touched with a glass rod. The low level of activity was maintained without detectable change for the next 14 hr. By the 15th through the 24th hr. movement was limited to 4 worms who could expand and contract their anterior ends, but could not move their posterior ends. Most animals were in the U-shaped position, but some curled into an oval position. At 42 hr. a few showed feeble movements, 4 were disintegrating, and the remainder were in the U-shaped position. Twenty-four hr. later all of the animals except 3 were disintegrating and only 1 was capable of any movement.

The second experiment was set up to determine if the worms are capable of acclimating to levels of salinity. The experiment was not designed to determine the range of acclimation to salinity, but to demonstrate that acclimation was possible. Fifty animals were placed in 75 per cent s.w. 96 hr. prior to testing. Survival times in 30, 40, 50, 60, and 70 per cent s.w. were determined for worms acclimated to 75 per cent s.w. and 100 per cent s.w. Ten animals were placed in each solution.

30 per cent s.w. All worms were immobile. In 2 hr., 7 of the group acclimated to 100 per cent s.w. and 3 acclimated to 75 per cent s.w. were sloughing epidermal cells. At 8 hr., all of both groups were disintegrating.

40 per cent s.w. All animals of both groups were immobile and failed to respond to normal stimuli. However, at 8 hr. 7 of the group acclimated to 75 per cent s.w. responded to a light stimulus from a no. 2 photoflood. Six of these were still active at 12 hr. and 5 showed feeble movements at 24 hr. None of the group acclimated to 100 per cent s.w. showed any response at any time.

50, 60, 70 per cent s.w. The number of animals surviving in each of these three concentrations is plotted against time in Figure 1. The curves for the animals tested in 50 per cent s.w.

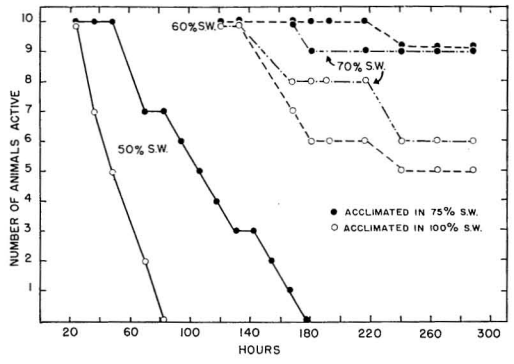

FIG. 1. Number of active animals plotted against time for *P. carmelensis* acclimated to 75 per cent s.w. and 100 per cent s.w. and tested in 50, 60, and 70 per cent s.w.

indicate that acclimation has taken place and has acted to increase survival time in the acclimated animals. The curve also indicates that the difference in survival time between the two acclimated groups increases with increasing time in the test environment. The curves also illustrate the variability in tolerance to salinity among individuals. This is particularly evident in the curves for the animals tested in 60 per cent s.w. and in 70 per cent s.w. After the least tolerant individuals of the worms acclimated to 100 per cent s.w. died, all the groups reached an equilibrium with essentially all the remaining animals surviving.

DIURNAL RHYTHM

The only known case of a persistent rhythm in the phylum Platyhelminthes has been reported for the acoel *Convoluta roscoffensis* (Harker, 1958). The worm comes to the surface of the sand at low tide and disappears into the sand when the tide returns. The rhythmic behavior is maintained in the laboratory for about 1 week in vessels of still water and is independent of day or night. *P. carmelensis* is found on the upper surfaces of algae or gravel during low tide and disappears into the gravel as the tide returns. The similarity between the two species makes desirable a study to determine if a diurnal or tidal rhythm occurs in *P. carmelensis*.

About 150 worms were collected near Pt. Pinos and brought into the laboratory. Ten animals along with a few pieces of rock were

placed in each of 10 plastic bowls. Two additional bowls were filled with gravel and 10 worms were placed in each. All the bowls were placed in an aquarium with the sides and top covered to keep out all light. In addition, the room was also darkened. Sea water from the laboratory system was circulated around the plastic bowls as a temperature control. The number of animals visible in each bowl was determined approximately every 2 hr. from 0800 until 2200 hr. for 2 days and spot-checked for 2 more days. The counts were made under a dim light.

During the first day there was a gradual increase in the number of worms that were visible. This leveled off by the morning of the 2nd day. The number of animals visible at the time of each count for the 2nd day is given in Table 1. There is no relationship between the number of animals visible and the time of day or the condition of the tide. Nor did the subsequent spot checks indicate any relationship. From this it is concluded that *P. carmelensis* does not show any diurnal or tidal rhythm of activity.

Observations in the field indicated that *P. carmelensis* came to the surface during low tide when the water was quiescent in the tide pools and when the light was relatively dim. If one waded through a pool in which the animals were located, one could observe the animals begin a downward movement. A similar downward movement was observed when the first waves of the incoming tide reached the pool. These observations indicated that water agitation was the stimulus for movement downward.

The following laboratory experiment tested the field observation. The data in Table 1 show that there was not much variation in the number of animals visible in the plastic bowls in the darkened aquarium. The number observed was particularly stable in the plastic bowls with gravel. Two bowls with rocks and two bowls with gravel were lighted sufficiently so that the animals could be counted. There was no reaction to the light in any of the dishes. Each of the bowls with gravel had 10 animals visible. One bowl was shaken gently; all the animals immediately became active and began crawling down into the gravel. After 3 min., only 2 worms were visible and both of them were crawling. All 10 animals remained visible in the control dish which was not shaken and only 1 animal was active. There were 5 worms on top of a rock in a third bowl. When this bowl was agitated, all the worms became active; 4 of them crawled under the rock within 2 min. The other animals in the bowl also became active, but could not move down as they were on the bottom of the dish. There was no activity in the fourth bowl, which also had a rock and served as an unshaken control to compare with the third bowl. From these experiments and from the field observations, it was concluded that *P. carmelensis* is negatively geotaxic in quiet water and positively geotaxic in agitated water.

TABLE 1

TOTAL NUMBER OF ANIMALS VISIBLE IN PLASTIC BOWLS WITH ROCK AND WITH GRAVEL
(Ten animals were placed in each bowl)

TIME OF OBSERVATION	NO. VISIBLE IN 8 BOWLS WITH ROCK	NO. VISIBLE IN 2 BOWLS WITH GRAVEL	TIME OF TIDE
0800	56	19	
			0810 low
1000	64	20	
1200	62	20	
1400	48	19	
			1444 high
1500	54	19	
1600	62	20	
2000	51	20	
			2105 low
2200	53	17	

During low tides when bright sunlight was present, *Polychoerus* was found under rocks and gravel, indicating that the genus might be positively geotaxic under bright light.

Twenty-one animals were distributed in six 25 × 75 mm. plastic vials so that there were at least 3 worms in each vial. The worms were allowed to come to rest after being added to the vials. The number of worms on the side of the vials was determined. Then the worms were stimulated for 3 min. with various intensities of light. All activity was recorded. The worms were kept in total darkness for 1 hr. between subsequent tests. The same worms were used throughout. The results are summarized in Table 2. Low light intensities had essentially no effect on the animals. Higher light intensities resulted in an increase in over-all activity with all animals becoming active at the highest intensity of light. Animals never crawled upward. There was an increasing percentage of animals on the sides of the vials that crawled downward with the increase in light. This experiment indicates that *P. carmelensis* becomes more active with increasing light intensity, and where directed movement is possible the animal crawls away from the source of light. Thus the absence of animals on the upper surface of rock and gravel during low tide and bright sunlight can be attributed to the reaction to light demonstrated above.

REACTIONS TO LIGHT

In the previous section some of the reactions of *P. carmelensis* to light were described. That experiment was designed to explain part of the upward and downward movement of the worms in the intertidal substrate in the absence of a diurnal rhythm. The reactions to light led to some further exploration of the behavior of the worms in relation to light.

The increase in the number of animals showing locomotion on the bottom of the vials at higher intensities of light (Table 2) indicated a photokinetic response. Photokinesis is usually defined as a change in the rate of undirected locomotion resulting from a change in the intensity of light. The photokinetic response in *P. carmelensis* was measured in two experiments.

The first experiment was designed to measure the rate of crawling under varied intensities of light. Two narrow strips of plastic were fastened 6 mm. apart in the bottom of a petri dish with a diameter of 14 cm. The plastic strips and the bottom of the dish were covered with black friction tape. A 5 cm. course was marked off between the plastic strips. The course was illuminated from one end. A worm was dropped into the dish at one end of the course with the light turned on, and the time that elapsed until the worm reached the end of the course was determined. The behavior of the worms was highly erratic. Some of them spent considerable time in turning the head from side to side, others ceased crawling before reaching the end of the course, and some crawled directly down the course. All of the worms were photonegative at the intensities used. Because of the variability in behavior, the experiment was discontinued after a small series of determinations were made. The rates of crawling for worms that crawled directly down the course illuminated by means of 5 ft. c. and 37 ft. c. were analysed by means of an analysis of variance and the between groups variance was statistically significant. The mean rate of travel was 0.86 mm/sec at 5 ft. c. and 1.34 mm/sec at 37 ft. c. The rate of crawling increased about 55 per cent when the light intensity was increased about 640 per cent. Similar slight increases in the rate of crawling with large increases in the intensity of light were found for *Dugesia gonocephala* and *Plagiostomum* sp. (Carthy, 1958: 37).

A second experiment attempted to measure photokinesis by determining the amount of activity initiated in a population of quiescent worms illuminated at various intensities of light. Five worms were placed in each of five petri dishes filled with sea water. After 1 hr., the worms were illuminated dorsally at various light intensities for 3 min. The time in seconds for a worm to respond was determined, as well as the nature of the response. Responses were of three types, (1) head raising; (2) body movement in which the animal might swing the anterior end side to side several times or show other changes in body form, but remaining essentially in the same location in the dish; (3) locomotion, in which the animal actively crawled about the dish. An activity index was determined

TABLE 2

REACTIONS OF *P. carmelensis* TO LIGHT OF VARIOUS INTENSITIES
(A total of 21 animals in 6 vials were tested for 3 min. at each intensity)

LIGHT INTENSITY ft. c.	NO. ON SIDES	NO. MOVING DOWN	PER CENT MOVING DOWN	HEAD MOVEMENT AT SURFACE	LOCOMOTION ON BOTTOM
1.7	15			1	
10.0	9			1	
37.0	8			3	2
130.0	11	4	36	3	5
225	8	7	87	1	12
400	5	5	100		16

by assigning an arbitrary value of 5, 10, and 20, respectively, to each of the above responses and dividing the sum of the values for individual worms by the sum of the number of seconds that elapsed until the animal responded or until 180 sec. elapsed. Animals that did not respond were included. This may be illustrated in the following equation:

$$\text{Activity index} = \frac{\text{sum of arbitrary values of response for all worms}}{\text{sum of number of sec. until response or until elapse of 180 sec. for all worms}}$$

The activity index will be higher if the number of animals responding to the stimulus increases or if the nature of the response is of a higher level or if both of these occur. The results are presented in Figure 2. Activity appears to be linearly related to light intensity over the ranges of light intensity studied. It is difficult to know if this relationship is a real one, for it might be an artifact of the method used to determine activity. However, the raw data indicate such a linear relationship, so that this relationship seems reasonably accurate. It probably does not exist at higher intensities, for once an animal has responded fully it can no longer respond to an increased stimulus.

On contrasting backgrounds, planarians come to rest on the darker ground (Ullyott, 1936). This reaction was tested in *P. carmelensis* in the following manner. Half the bottom, the sides, and the upper edge of the sides of a 4½-in. petri dish were painted black and the other half of the bottom was painted white. The dish was half-filled with sea water and placed in a metal trough through which sea water circulated to maintain a relatively constant temperature and illuminated from above by 15 ft. c. of light. Ten (in some experiments, 20) animals were added to the petri dish as close to the center as possible. The animals were allowed 1 hr. to come to rest, after which the animals on each background were counted. The dish was rotated 90° between trials so that if the animals were reacting to light being reflected from the wall of the room or from the sides of the dish, such a directional orientation could be detected. The position of rest of each animal for each trial was plotted on a drawing of a petri dish. There was no evidence of directional orientation in any of the experiments. Forty different individuals were used in the first set of experiments. All animals were kept in the dark between trials. Any animal that crawled up on the side of the dish was considered to be on a black background. The experiments extended over 5 days. Of 40 animals tested, 33 came to rest on the white background and 7 came to rest on the black background. The marked orientation to the white background (chi square $= 16.8$, $p < 0.001$) was unexpected in view of the results with planarians. The testing was repeated four times using the same animals. The results were similar in all cases. Since all of the animals came from an area of broken shell, most of which was white, it was postulated that the animals were acclimated to the white background. If this hypothesis were correct, then it should be possible to acclimate the worms to a black back-

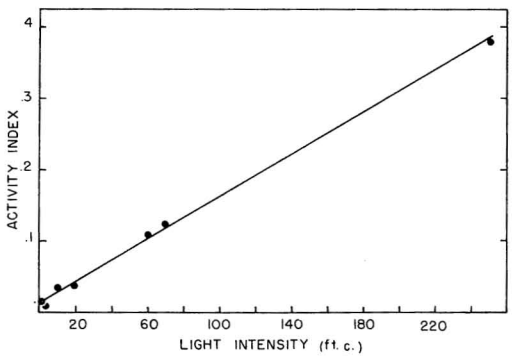

FIG. 2. The activity index of a population of *P. carmelensis* plotted against light intensity. Curve fitted by eye.

ground. Twenty animals were kept under constant light in a black plastic bowl for 96 hr. The animals were tested at 24, 72, and 96 hr. At 24 hr., 12 animals oriented to white and 8 to black, showing no significant difference (chi square $= .8$, $p < 0.5$), 72 hours, 8 oriented to white and 12 to black, again showing no significant difference. At 96 hr., 5 oriented to white and 15 to black, now showing a significant difference (chi square $= 5.0$, $p < 0.05$). The animals were tested again 2 days later. During the two days, they were kept on the black background but received light only from 0800 to 1800 hr. Several tests were run, with the animals being kept in darkness between tests. In the first test, 5 animals stopped on the white background and 15 on the black background. The marked orientation to the black background was significant (chi square $= 5.0$, $p < 0.05$). However, when the test was repeated using the same animals, two of the tests gave essentially the same results, but the third showed no difference in background selection.

A second series of tests for background choice was made with newly collected worms from Carmel Point. Forty worms were placed in black dishes and 40 were placed in white dishes. The worms were kept under constant light and tested after 96 hr. The testing extended over 2 days and the animals were kept in darkness during the period of testing. The animals were tested under 35 ft. c. of light. The animals kept on a white background went to the white side of the dish 27 times and to the black side 11 times. The selection of the white background is highly significant (chi square $= 6.7$, $p =$ c. 0.01). The animals kept on the black background went to the white side of the dish 9 times and to the black side 29 times. The selection of the black background is highly significant (chi square $=$ 10.5, $p < 0.01$). Each of these tests was repeated once using the same animals. Results were essentially identical.

The animals were kept in their respective dishes and given 48 hr. of constant light. They were then placed in the dark and subsequently tested under 70 ft. c. of light.

The animals kept on a white background went to the white side of the dish 21 times and to the black side 16 times. There is no indication that background selection occurred (chi square $= 0.66$, $p < 0.5$). However, the animals kept on a black background went to the black side of the dish 33 times and to the white side 7 times. Selection of the black background was significant (chi square $= 16.0$, $p < 0.001$). Each of these tests was repeated twice using the same animals. The worms acclimated to the black background showed about the same pattern, but with lowered chi-square values. The animals acclimated to the white background showed almost complete randomness in selection of background (chi square $= 0.01$).

These is no ready explanation for the shift in background selection by the worms acclimated to the white background. The experiments concerning photokinesis demonstrated that the worms had a differential sensitivity to light, some reacting to a weak stimulus, others to a strong stimulus. Because of the variation in sensitivity to light, it seems reasonable to postulate that under the increased light intensity, photokinesis was stimulated more in the light-sensitive animals. These sensitive animals then oriented to the black background to reduce the amount of stimulation. The less sensitive animals continued to orient to the white background to which they were acclimated. That there is a threshold of sensitivity to light whereby the reaction to light stimulation is reversed is indirectly indicated. Costello and Costello (1938*b*) reported that *P. carmelensis* may "... be positively phototropic to moderate light intensities"; the positive phototropism was

evidenced by the gathering of the worms on the lighted side of the aquarium in which they were kept. Taxic reactions were not studied as such in this series of experiments, but the experiments on diurnal rhythm demonstrated that the animals were negatively phototaxic at high light intensities and showed no response at low intensities. Thus the possibility of a differential response to low and high light intensities exists, as has been found for other animals (Clarke, 1930; Baylor and Smith, 1957). Clarke (1932) found that a change of illumination must rise above a certain threshold to be effective in causing a reversal of phototropic signs in *Daphnia.*

It was mentioned previously that worms acclimated to a black background showed a lesser degree of choice of the black background when the tests were repeated. Since the animals were kept in darkness except while being tested, it seemed possible that some of the worms were losing their acclimated condition and perhaps were moving in a more random manner. Therefore, both the animals acclimated to a white background and the animals acclimated to a black background were illuminated with 70 ft. c. for 12 hr., placed in darkness for 12 hr., and then tested. Thirty of the animals acclimated to the black background came to rest on the black background while 8 selected the white background. The orientation to the black background was highly significant (chi square $= 12.6$, $p < 0.001$). Nineteen of the animals acclimated to the white background came to rest on the white background and 19 selected the black background. Thus it was not possible to condition the animals to select the white background under 70 ft. c. of light under the conditions of the experiment. However, the animals acclimated to the black background responded almost to the same degree as in the original test. This experiment suggests that failure to maintain an orientation to a white background at 70 ft. c. is a result of animals more sensitive to light changing their orientation from the white background to the black background.

FEEDING BEHAVIOR

Five *P. carmelensis* were placed in a Syracuse watch glass with a dozen copepods, *Tigriopus californicus.* One of the copepods came to rest near the left anterior end of a *Polychoerus* which had stopped crawling. The anterior end of the worm was raised and with a sudden whiplike movement it was brought down over the *Tigriopus.* The worm assumed a cup-shaped position over the copepod. The copepod was quickly engulfed and its movements inside the body of the worm could be observed. These movements continued for 10 min. The capture of the crustacean by *Polychoerus* was similar to the manner of prey capture by the acoel *Convoluta paradoxa* (Jennings, 1957). Dead *Tigriopus* were not ingested.

CONCLUSIONS

1. *P. carmelensis* can tolerate salinity conditions ranging from 75 to 125 per cent s.w. indefinitely. Worms were quickly inactivated at concentrations of sea water above and below these values. Animals kept in 50 per cent s.w. for 5 hr. and transferred to 100 per cent s.w. recovered normal activity by 16 hr. after transfer.

2. *P. carmelensis* was acclimated to 75 per cent s.w. and survival time was increased at the range of salinities tested over controls acclimated to 100 per cent s.w.

3. There was no evidence of a diurnal or tidal rhythm of activity. Worms tended to be negatively geotaxic in quiet water at low light intensities and positively geotaxic in agitated water or at high light intensities.

4. Photokinesis, measured as the amount of activity in a population of worms, was linearly related to light intensity over the range of light intensities used. Only slight differences were found in the rate of crawling of worms over a measured course under highly different intensities of light.

5. At 15 ft. c. light intensity, worms collected from tide pools with white shell and rock chose the white background when placed in a petri dish with half the bottom painted white and the other half black. Worms were acclimated for 96 hr. in dishes painted black and in dishes painted white. The black-acclimated worms chose the black background and the white-acclimated worms chose the white background when tested in the petri dish with contrasting backgrounds of black and white. The reaction was

highly significant at 15 ft. c. and 30 ft. c. However, at 70 ft. c., the worms acclimated to a white background showed no preference when tested on contrasting backgrounds. The worms acclimated to the black background continued to orient to the black background when tested on contrasting backgrounds. It was postulated that the change in response at 70 ft. c. of animals acclimated to the white background was a result of crossing a threshold of light sensitivity so that the more sensitive animals tended to orient to the black background while the less sensitive animals tended to orient to the white background.

6. The capture of a copepod prey is described.

REFERENCES

BAYLOR, E. R., and F. E. SMITH. 1957. Diurnal migration of plankton crustaceans. In: Recent Advances in Invertebrate Physiology, B. T. Scheer, ed., Univ. of Oregon Publications. 304 pp.

CARTHY, J. D. 1958. An Introduction to the Behavior of Invertebrates. Macmillan Co., New York. 380 pp.

CLARKE, G. J. 1930. Change of phototropic and geotropic signs in *Daphnia* induced by changes in light intensity. J. Exp. Biol. 7: 109–131.

——— 1932. Quantitative aspects of the change of phototropic sign in *Daphnia*. J. Exp. Biol. 9: 180–211.

COSTELLO, H. M., and D. P. COSTELLO. 1938*a*. Copulation in the acoelous turbellarian *Polychoerus carmelensis*. Biol. Bull. 75: 85–98.

——— and ——— 1938*b*. A new species of *Polychoerus* from the Pacific Coast. Ann. Mag. Nat. Hist. ser. 11, 1: 148–155.

——— and ——— 1939. Egg laying in the acoelous turbellarian *Polychoerus carmelensis*. Biol. Bull. 76: 80-89.

DE BEER, G. R. 1954. The Evolution of the Metazoa. In: Evolution as a Process, Huxley, Hardy, and Ford, ed., George Allen and Unwin, London. 367 pp.

HANSON, E. D. 1958. On the origin of the Eumetazoa. Systematic Zool. 7: 16–47.

HYMAN, L. H. 1951. The Invertebrates: Platyhelminthes and Rhynchocoela. McGraw-Hill Book Co., New York. 550 pp.

——— 1959. The Invertebrates: Smaller Coelomate Groups. McGraw-Hill Book Co., New York. 783 pp.

JENNINGS, J. B. 1957. Studies on feeding, digestion and food storage in free-living flatworms (Platyhelminthes: Turbellaria). Biol. Bull. 112: 63–80.

RICKETTS, E. F., and J. CALVIN. 1952. Between Pacific Tides. 3rd ed., rev. by J. W. Hedgpeth. Stanford University Press, California. 502 pp.

ULLYOTT, P. 1936. The behaviour of *Dendrocoelum lacteum*, II. Responses in non-directional gradients. J. Exp. Biol. 13: 265–278.